Localization of Radioisotope (Chlormerodrin Hg-203) in Experimental Cerebral Infarction

BY ALDEN W. DUDLEY, JR. MD.,* STEVEN LUNZER, M.D.,† AND ALBERT HEYMAN, M.D.†

Abstract:
A combined histological and radioautographical study was made to determine the mechanism for the selective localization of Hg-203-labelled Chlormerodrin in the brain of 13 cats with experimentally induced cerebral infarction.

The presence of the radioactive tracer in the infarcted areas was noted within a few days after the onset of the lesions and was increased by hemorrhage into the tissues. The radioautograms revealed the radioactive material to be localized mostly within macrophages and in areas surrounding capillary proliferation. These two pathoanatomic processes associated with necrosis of the cerebral tissues most likely account for the abnormal accumulation of radionuclide tracer in brain scans of patients with cerebral infarction.

ADDITIONAL KEY WORDS hemorrhagic cerebral infarction radioautograph neuropathology brain edema growth of capillaries

Although radioisotope scanning of the brain has been found to be a useful technique for the diagnosis and localization of cerebral infarction,1–3 little is known about the mechanisms underlying the selective concentration of the tracer within the infarcted tissues. There is, however, a definite relationship between the age of an infarct and the degree of uptake of the radionuclide. The brain scan is usually normal early in the course of cerebral infarction; it may become abnormal during the second week. After three to six weeks, the uptake of the radioactive material by the infarcted tissues usually diminishes and the brain scan ordinarily reverts to normal.

It has been suggested that the pattern of isotope uptake is related to various pathologic processes such as breakdown of the "blood-brain barrier," necrosis, edema, or glial reaction. The localization of the isotope in cerebral lesions has been determined histologically in only a few instances,4–6 and not at all in infarcts.

In the present study, cerebral infarction was produced experimentally in cats, and the localization of Chlormerodrin Hg-203 in the brain was determined by combined histological and radioautographical techniques. An attempt was also made to correlate the extent of cerebral uptake of the radioactive compound with the duration and severity of cerebral infarction.

Methods
Cerebral infarction was produced in 13 adult cats by ligation of the middle cerebral artery using a modification of the surgical technique developed by Sundt and Waltz.7 A left-sided craniectomy was made after resection of the zygomatic arch and the coronoid process of the mandible. The dura was opened and the brain lifted to expose the middle cerebral artery. Mayfield clips were applied across this vessel close to its origin.

*Department of Pathology, University of Wisconsin, Madison, Wisconsin, 53706.
†From the Divisions of Neuropathology and Neurology and the Center of Cerebrovascular Research, Duke University School of Medicine, Durham, North Carolina.
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References:
After the operation, the animals were kept under observation for periods ranging from two to 72 days, following which they were given an intravenous injection of 5 \( \mu \)g per pound of body weight of Hg-203-labelled Chlormerodrin. Two hours later, the animals were sacrificed and the brain immediately removed for formalin fixation. Two to seven days later, each cerebral hemisphere was cut into eight blocks, using one sagittal and three coronal sections. The gross appearance of the infarct was noted when the brain was removed and after sectioning. Each section of cerebral tissue was weighed; its content of radioactivity was measured with a Packard scintillation well counter and recorded as counts per minute per gram of tissue. The block of infarcted tissue with the greatest radioactivity was retained along with the corresponding section of the normal hemisphere, and both were studied with radioautographical and histological methods.

Ten serial sections (4 \( \mu \)g each) were prepared from each block of tissue and alternate sections were used for radioautographical and histological examinations. The radioautographical emulsion was applied at 50°C to secure as thin a film of emulsion as possible. The incubation time for the radioautographs was three, five, seven, or 14 days. Cells of particular interest noted on the radioautographs were identified in the alternate sections which were stained with Luxol fast blue-hematoxylin and eosin. Additional sections were set aside for special stains for glial cells (Holzer iron) (Turnbull blue), etc. All sections were examined by routine light microscopy and a split-image microscope was used to obtain direct comparison of the infarcted and contralateral normal cerebral tissues.

**Results**

Following the surgical procedure, two of the cats showed no neurological deficit. Three other cats had minimal abnormal signs consisting of a tendency to circle toward the side of the infarct. Four others had definite weakness and spasticity of the contralateral limbs, but were able to walk. There also was evidence of loss of pain sensation in the affected extremities. The remaining four animals had hemiplegia and could not stand or walk. Each of the 11 animals with neurological abnormalities showed slight to moderate improvement within a few days or weeks after the operation.

The animals were sacrificed at predetermined time intervals and the brains examined. In five animals, there was an area of softening, with loss of cortex and centrum ovale in the distribution of the peripheral branches of the middle cerebral artery. Petechial hemorrhages were sometimes present. In seven cats, the infarcted area was hemorrhagic and included the basal ganglia (fig. 1). In these animals, the lesion was apparently due to involvement of the perforating branches as well as the peripheral branches of the middle cerebral artery. The brain in the remaining cat showed no gross changes, but microscopic lesions were demonstrable. This animal had no neurological abnormalities following the surgical operation. In general, the animals with the most severe signs had the largest areas of infarction.

Cerebral uptake of Chlormerodrin Hg-203 varied widely, but in a given animal the amount of radioactivity in each section of the normal hemisphere was relatively constant. The selective uptake of the radioactive tracer in sections of the tissue was expressed as the ratio of counts per minute per gram of the infarcted hemisphere to that of the contralateral normal hemisphere. The results are plotted in figure 2. A correlation appeared to exist between the degree of radioactivity in the infarcted tissues and the duration of the lesion.

Transverse section of brain five days after surgery shows operative hemorrhage and striatal ischemic necrosis.
RADIOISOTOPE IN CEREBRAL INFARCTION

Ratio of radioactivity in infarcted tissues to activity in contralateral cerebral tissues of the same animal. Each bar represents one animal. The animal with the highest ratio had a moderately severe hemorrhage in the lenticular nucleus.

Cats with cerebral infarction of two days' duration had three to five times as much radioactivity in the infarcted tissue as normal brain sections. In contrast, animals with infarction of five days' duration showed as much as 40 times as much tracer in the infarcted tissue as in the contralateral hemisphere. Infarcted tissues of 7 to 35 days' duration showed progressively less radioactivity.

Microscopic examination of the infarcted tissue revealed a sequence of cellular reactions which could be related to localization of the tracer material. Infarcts of two days' duration were characterized by perivascular hemorrhage and evidence of eosinophilic transudate (fig. 3). Swelling of myelin and infiltration of polymorphonuclear leukocytes were also noted, but no macrophages were seen. The amount of radioactivity at this stage of infarction as seen in the radioautographs did not appear to be greater than that found in the tissues of the normal hemisphere.

Infarcts of five days' duration showed evidence on microscopic examination of hemorrhage at the periphery of the lesion with an

Section of two-day infarct showing perivascular hemorrhage and proteinaceous transudate into perivascular space. (Luxol fast blue—H&E, X250.)

Section of five-day infarct with macrophages containing red blood cells and myelin debris. (Luxol fast blue—H&E, X250.)

Section of five-day infarct shows endothelial proliferation of capillaries. (Luxol fast blue—H&E, X250.)
accumulation of macrophages containing degenerating red blood cells and myelin (fig. 4). Marked proliferation of capillaries at the margin of the infarct was also noted (fig. 5). Radioautographs of the lesion at this stage of development revealed increased concentration of the tracer material within macrophages and on and between red blood cells in the areas of hemorrhage. An increase in radioactive material was noted, particularly at the site of capillary proliferation where it was distributed throughout the neuropil with no definite increase within cells or vessels. Histological preparations of this area consisting of 20 μ radioautographs showed a heavier concentration of the tracer substance in the perivascular and tissue space than in the vascular lumen or capillary wall.

In infarcts of seven days' duration, tissue necrosis and dissolution were obvious but capillary proliferation was somewhat diminished. The line of demarcation between the necrotic and viable tissue was well defined and there was early cyst formation with a glial lining. The radioactive material persisted at the areas of increased vascularity and within macrophages.

From the ninth to the twenty-second day, the neurons in the area of reversible injury showed evidence of radioactive material. The radioautographs, which were made after prolonged exposure, particularly showed an increase in neuronal radioactivity (fig. 6). Iron stains of the neuronal areas were negative.

In the infarcts of 14 days' duration, the cyst wall was well outlined and macrophages containing myelin partially filled the lumen. These macrophages continued to contain radioactive material. By the third to fifth week, the cystic lesion showed greater maturity with a decreasing number of macrophages and a more complete glial lining. The subependymal margin of tissue was spared. The affinity for the radioisotope material was markedly diminished

FIGURE 6
Section of 14-day infarct shows radioactive material associated with neurons at viable margin. (Luxol fast blue—H&E, ×840.)
at this time. In the animal showing a 72-day-old infarction of the brain, the margins of the cyst were lined by a dense matrix of astrocytes and there was only a minimal increase in the radioactivity at the site of the lesion as compared to normal sections.

Discussion
Our findings on the uptake of Hg-203-labelled Chlormerodrin in the brains of cats with experimentally induced infarction support our previous observations on the clinical use of brain scans in cerebrovascular disease. Serial brain scanning in patients with cerebral arterial occlusion showed that a significant uptake of radioactive material did not usually appear in infarcted tissues until after the first week of the illness. In animals with experimental infarction, the greatest degree of uptake of the radioactive material appeared slightly earlier, approximately five days after the onset of the lesion. In patients with cerebral infarction, the abnormalities in the brain scan became less severe after three weeks and usually disappeared completely after a month or two. Similarly, in the experimental lesions, the uptake of the radionuclide was progressively less evident in infarctions of three to five weeks' duration.

The histological changes in experimental cerebral infarction were of considerable interest. An orderly sequence of tissue reaction to ischemia was noted and appeared similar to that found in man. The initial histological change consisted of perivascular edema with diapedesis of red blood cells and neutrophils at the periphery of the infarct. The center of the lesion developed evidence of necrosis. The most dramatic changes appeared about the fifth day after onset of the lesion and were characterized by endothelial proliferation in the peripheral capillaries with migration of macrophages to the center of the necrotic area. Progressive lysis and ingestion of the necrotic debris by the macrophages led to the development of a cyst which became lined with astrocytes and traversed by bridging capillaries and glial bands. During the acute phases of the lesion, the neurons at the viable margins of infarction showed central chromatolysis. In the subacute and chronic stages, the surviving neurons showed apparent recovery with improvement in their morphological appearance.

The patho-anatomic basis for the accumulation of radioactive substances in infarcted cerebral tissues was elucidated by the combined histological and radioautographical methods. An increase in permeability of the vessels in various lesions of the brain is generally considered to be an important factor in the localization of radionuclides. In our animals with ischemia of two days' duration, however, the defects in the vessel wall associated with perivascular hemorrhage and transudate were not accompanied by a significant accumulation of the radioactive Chlormerodrin.

Edema of the cerebral tissues likewise does not appear to be a major factor responsible for development of the abnormal brain scan. Brain swelling occurs most often within a few days after the onset of infarction, at which stage brain scans of patients are usually negative. In the animals with experimental infarction, the radioactive material is minimal in the edematous spaces within the brain and in the perivascular areas. The highest concentration of radionuclide occurred in the areas of new capillary formation and within the macrophages infiltrating the necrotic cerebral tissues. Both of these histological phenomena appeared about the fifth day after onset of the cerebral infarction and persisted during dissolution and repair of the lesion. It seems likely that the increased uptake of the radionuclide noted in the brain scans of patients with cerebral infarction is related primarily to these patho-anatomic processes, i.e., macrophage formation and development of new capillary vessels. The persistence of abnormal brain scans for several weeks in patients with severe necrosis of cerebral tissues would also be consistent with the continuous presence in such lesions of macrophage infiltration and neocapillary proliferation. The increased uptake of radioactive material about the newly formed capillaries suggests that these vessels may have altered permeability.

The radioautograms in the experimental animals showed unexpected localization of the radionuclide associated with neurons in the marginal areas of the infarcted tissues. The histological appearance of these cells resembled those with iron deposits, but stains for this metal were negative. Whether the radioactive tracer was encrusted on the surface or within these cells could not be determined from our
tissue preparations. In all probability, the presence of the radionuclide was a surface phenomenon related perhaps to alterations in the neuronal membrane associated with ischemia. This condition may be analogous to ferrugination of neurons sometimes seen in cerebral infarction.

The histological changes in animals with cerebral infarction correspond generally with those found in humans with lesions at similar stages of development. It seems reasonable to assume, therefore, that the radioautographical findings in the animals are also comparable to those occurring in brain scans of patients with cerebrovascular disease. Although use of radioactive Chlormerodrin in clinical brain scanning has been replaced in many hospitals by tracers with shorter half-lives, such as Technetium$^{99m}$, the mechanisms for the abnormal localization of radionuclides in injured cerebral tissues are probably similar.

The infarcted cerebral tissues in some of our animals contained considerable hemorrhage perhaps caused by operative trauma. The radioactive uptake in such lesions appeared to be greater than that seen in ischemic infarcts. There is some evidence to indicate that hemorrhage infarcts in humans also show more rapid accumulation of radionuclides and may account for the early appearance of abnormal brain scans in some patients with cerebrovascular disease, particularly those with cerebral embolism.

References
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